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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Overexpression of ErbB2 in mammary epithelial cells increases activity, but not expression, of c-src. This effect is not mimicked by other transforming oncogenes. These results suggest that previous results in transgenic animals are likely to be due to direct effects on mammary epitheliaum, not to alterations in systemic physiology (such as hormone levels) or to altered epithelial stromal interactions. Results indicate that induction of c-src activity is mediated by increased activity of a src-directed phosphatase that removes an inhibitory phosphate at Y527. SHP2 appears to be a leading candidate for the phosphatase. In addition, the activation of c-src by ErbB2 appears to play a critical role in inducing a tumor phenotype. These results that strategies to modify src activity or the activity of the src-directed phosphatase(s) may prove useful in modifying tumor progression.

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INTRODUCTION

The hypothesis of this proposal is that ErbB2 expression regulates the activity of c-src and that this activation is a factor in mediating ErbB2 induced tumorigenesis. Previous work with human tumors has shown increased c-src activity (Ottenhoff-Klaff et al., 1992). Tissue from mice overexpressing ErbB2 show increased activity of c-src (Muthuswamy et al., 1994). Knockout mice lacking c-src also exhibit decreased tumorigenesis induced by polyomavirus middle T antigen (Guy et al., 1994). Data from our laboratory and others (Zhair et al., 1993) show that non-tumorigenic mammary epithelial cells are readily transformed to a tumorigenic phenotype by overexpression of ErbB2. This tumorigenic transformation is accompanied by increased activity of c-src (see below).

The overall project has two major areas of focus: the mechanism by which ErbB2 activates c-src and the consequences of c-src activation. The first year's work has focused on the activation of c-src by ErbB2. There are several ways by which ErbB2 could regulate c-src activity: by altering c-src expression levels, by decreasing expression and/or activity of csk, by increasing expression and/or activity of c-src-directed phosphotyrosine phosphatases or by phosphorylation-independent activation of c-src.

As described in the STATEMENT OF WORK the goals of this project were to:

I. Verify ErbB2 overexpression activation of c-src and map phosphorylation sites of src

II. Determine if src activation by ErbB2 is correlated with altered activity of src kinases or phosphatases

III. Evaluate the effect of inhibiting c-src activity on ErbB2 mediated tumorigenesis

BODY

Experimental Methods

Cell Lines

Parental Lines

The nontumorigenic human mammary epithelial cell line 184.A1 and the nontumorigenic mouse line NMuMG was used in most studies covered by this report. Cells were routinely grown in DMEM:F12 + 10% FBS, 5 μg/ml insulin and 10 ng/ml EGF.

ErbB2, Ras and src transformed lines

Cells were transfected by ErbB2, v-Ha-ras and activated src (Tyr527 mutated to Phe) in vectors containing a G418 resistance marker. Vectors were cloned in DH5α E. coli using standard procedures. Cells ($5x10^6$ in 0.5 ml HBSS) were placed in electroporation cuvets with 2 mm electrode space and pulsed with 1.2 kV/cm field strength. Cells were left on ice for 10 minutes, then returned to culture media. After 24 hours, media was changed to contain 400 μg/ml G418 sulfate and selection continued for 4 weeks.

ErbB2 and dominant negative src or dominant negative SHP2 transfected lines

For studies on the functional consequences of c-src activation by ErbB2, cells were initially transfected with dominant negative c-src under control of the CMV promoter and in a neomycin resistance vector (Bell et al., 1992) or with empty vector. To examine the role of the SHP2 phosphatase, similar studies were performed with a dominant negative SHP2 construct (Serividei et al., 1998). These cells were then selected for stable transfection by selection in media containing G418 to give control and dominant negative c-src transformed cells.

The ErbB2 was then excised from the initial neomycin resistance vector and subcloned into a Zeocin resistance vector. Control and dominant negative c-src cells (above) were then transfected with this vector or with the empty vector and selected in media containing G418 and Zeocin.

Characteristics of cell lines

ErbB2 expression

To assess overxpression of ErbB2, cells were plated in 60 mm culture dishes (10^7 cells per dish and grown for 24 hours. Media was removed and SDS loading buffer lacking mercaptoethanol and bromophenol blue added to plates. Cells were scraped into tubes and heated to 95°C for 5 minutes. Protein content was then assessed by BCA assay (Pierce Chemical Co., Rockford, IL) and equalized among samples. Bromophenol blue and 2-mercaptoethanol were added to samples, equal protein was then separated by SDS-PAGE using a 7.5 % separating gel (Laemmli, 1970) and transferred to PVDF membranes (Towbin et al., 1979; Fenton and Sheffield, 1993). Membranes were probed with anti-ErbB2 (Transduction Laboratories, Lexington, KY) and detected with chemiluminescence (DuPont, Boston, MA) as described previously for other proteins (Fenton and Sheffield, 1993). Band intensity was quantitated by computer assisted densitometry (Collage®, Fotodyne, New Berlin, WI).

Growth on soft agar

Cells (10^4) were suspended in 2 ml of 0.3% agarose dissolved in culture media and layered onto 2 ml of hardened 0.5% agarose in culture media. After 10 days, cells were observed for growth.

Growth in nude mice

Inguinal (fourth) mammae of 3 week old athymic mice were exposed and epithelial containing portions excised. Cell suspension (10 µl of a suspension of $1x10^8$

cells/ml in HBSS) were injected into the resulting fat ape and incisions closed with wound clips. Mice were observed daily for tumor formation and euthanized with 5 mg pentobarbital i.p. when tumors were palpable. Tumors were processed for histological evaluation by fixing in phosphate buffered formalin, embedding in plastic, cutting into 5 μm thick slices, mounting on slides and staining with hematoxylin and eosin.

Measurement of c-src activity

Cells were lysed with lysis buffer (30 mM sodium pyrophosphate, 10 mM EDTA, 50 mM NaCl, 50 mM NaF, 1% Triton X-100, 0.1% BSA, 1 mM sodium orthovanadate, 1 mM PMSF). Lysates were clarified by centrifugation for 10 minutes at 15,000 g, protein content determined by BCA assay and protein equalized among samples. Samples were then incubated for 2 hours at room temperature with anti c-src and agarose conjugated protein A and G. Samples were centrifuged and beads washed 4 times with lysis buffer. Beads were then incubated with kinase buffer (10 μl of 200 mM HEPES, pH 7.0 containing 125 mM $MgCl_2$, 25 mM $MnCl_2$ and 0.25 mM sodium orthovanadium. Substrate (5 μl of a 0.5 mM solution of [lys^{19}]cdc2(6-20) was added and reactions started by adding 5 μl of γ^{32}P-ATP (0.5 mM, specific activity of 5,000 dpm/pmole). After 5 minutes, reactions were stopped by adding 10 μl of 50% acetic acid and samples were centrifuged (5,000 g for 5 minutes). Supernatant was spotted onto Whattman P81 phosphocellulose paper, washed 4 times with cold 100 mM phosphoric acid, rinsed with acetone, dried and counted by liquid scintillation. In addition, reactions were performed without peptide or with [$val^{12}ser^{14}lys^{19}$]cdc2(6-20) (which should not be phosphorylated by src) as a substrate. c-src content of immunoprecipitates was determined by western blot analysis, essentially as described above for ErbB2 and resulting data used to correct for any differences in src content.

Measurement of c-src phosphorylation

C-src was immunoprecipitated as described above. Src was then digested with 50 mg/ml cyanogen bromide in formic acid and lyophylized. Resulting peptides were separated by tricine PAGE (van der Geer et al., 1993) and transferred to PVDF membranes by electroblotting. Membranes were then blocked and probed with anti-phosphotyrosine as previously described (Fenton and Sheffield, 1993). Density of bands was determined by densitometry (Collage®). In addition, ErbB2 was immunoprecipitated, separated by PAGE and associated src excised and processed as described above.

Measurement of c-src Expression

Cells were lysed as described above for ErbB2 expression. Protein content of lysates was equalized, proteins separated by SDS-PAGE (12% separating gel) and probed with anti-c-src as above.

Measurement of other src-related kinases

Other members of the src family, including lyk, lyn and fyn, were immunoprecipitated as described for c-src and activity estimated essentially as described for c-src.

Measurement of CSK content

Cells were lysed with SDS loading buffer and proteins separated by SDS-PAGE. Proteins were transferred to PVDF membranes, blocked with BSA and probed for CSK by Western blot analysis using chemiluminescence detection. Computer assisted densitometry was used to estimate relative band intensity.

Measurement of CSK activity

Cells were lysed with lysis buffer (50 mM HEPES containing 1 mM PMSF, 40 mM Sodium orthophosphate, 1 mg/ml BSA and 1% Triton X-100), clarified by centrifugation (14,000 g for 15 minutes) and CAK immunoprecipitated from lysates using a rabbit

polyclonal antibody and agarose conjugated protein A/G. Beads were washed with lysis buffer and then incubated with assay buffer (200 mM HEPES containing 100 mM $MgCl_2$, 25 mM $MnCl_2$, and 0.1 mM Na_3VO_4) with substrate peptide corresponding to the C-terminus of src (TSTEPQY(PO_4)QPENL). Assays were begun by adding γ^{32}P-ATP (10 μM containing 250,000 dpm) and continued for 5 minutes. Assays were stopped by adding 50% acetic acid and 1 mg/ml BSA. Tubes were then centrifuged (14,000 g for 5 minutes) and supernatant spotted onto Whatman 3MM filter Paper. Filter paper was washed four times with 100 mM phosphoric acid, dried and counted by liquid scintillation. Background was estimated by performing the assay in the absence of substrate peptide and was subtracted to give substrate-dependent activity.

Measurement of phosphatase activity

For assays on crude cell lysates, cells were lysed with 50 mM HEPES containing 40 mM sodium pyrophosphate, 50 mM NaF, 0.1 mM Na^3VO^4 , 1 mM PMSF and 1% Triton X-100. Lysate was concentrated with an Amicon centrifical concentrator and resuspended in phosphate-free buffer. Protein content was determined by BCA method (Pierce Chemical Co., Rockford, IL), equalized among samples and phosphatase activity assessed by the Malacite Green method of assessing free phosphate (Harder et al., 1994) and using a peptide corresponding to the C-terminus of src (peptide 301, Biomole, Plymouth Meeting, PA) or a peptide corresponding to the autophosphorylation site of src (peptide 312, Biomole) as substrate peptides.

For membrane preparations, cells were lysed as described above except that the buffer lacked Triton X-100, centrifuged (2000 g for 5 minutes) and supernatant centrifuged at 50,000 g for 60 minutes. Pellets were dissolved in phosphate free assay buffer containing 1% Triton X-100 and phosphatase activity assessed as described above. Cytosol was assessed as described above for cell lysates.

To assess phosphatase associated with ErbB2, ErbB2 was immunoprecipitated and resulting immunoprecipitates assayed as described above.

Western Analysis of phosphatase activity

Cell lysates, membranes or ErbB2 immunoprecipitates were prepared as described above. Proteins were separated by SDS-PAGE, transferred to PVDF membranes and western blots probed with antibodies to the indicated phosphatases. Computer densitometry was used to assess relative band intensity.

Results

Characterization of cell lines

ErbB2 expression

Cells transfected with ErbB2 exhibited substantially greater concentrations of ErbB2 than parental cells, indicating that the ErbB2 transfected lines dramatically overexpressed the gene at the level of cell protein content.

Growth on soft agar

ErbB2 and ras transformed cells grew readily on soft agar, whereas the parental cells were incapable of growing on soft agar. This, together with nude mouse tumor formation (below) suggests that the cells are tumorigenically transformed.

Growth in nude mice

ErbB2 and ras transformed cells were capable of forming tumors in nude mice (100% of inoculated mice had palpable tumors within 30 days). The tumors were typical of poorly differentiated breast adenocarcinomas. No tumors were formed by the parental cells, suggesting that ErbB2 and ras induced tumorigenic transformation.

c-src activity

Src activity (Figure 1) was dramatically increased by ErbB2 transformation, but only modestly increased by ras transformation. When corrected for amount of c-src in immunoprecipitates, essentially identical results were obtained, as c-src content of immunoprecipitates exhibited little difference among treatments. This would be consistent with a model in which ErbB2 induces c-src activity by activating existing enzyme, rather than inducing c-src expression.

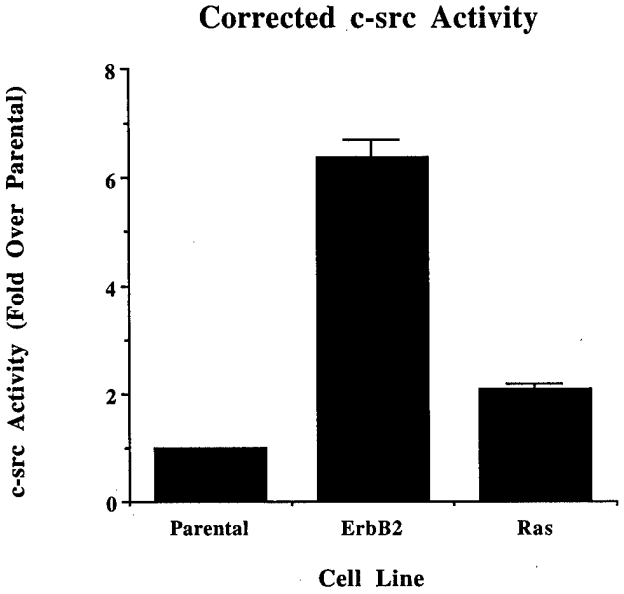

Figure 1. c-src activity in 184.A1 cells or 184.A1 cells transfected with ErbB2 or ras. Values reported after division by relative amount of c-src in the immunoprecipitate in order to give a specific activity change. Mean ± SEM of 4 observations.

Linearity of assay over time was verified by conducting assays for 2, 4, 6 or 8 minutes. Results indicated that assay was linear over time. The c-src kinase assay used above was found to be dependent of added substrate peptide. Omission of the peptide or use of a peptide not phosphorylated by c-src yielded essentially background activity, as did omission of the enzyme. Diluting the enzyme preparation used in kinase assays caused a corresponding decrease in kinase activity, indicating that the results were linear over the enzyme concentration range used in the studies. Performing the assay using different starting concentrations of substrate peptide indicated that the results obtained

were not an artifact of substrate concentration. ATP concentration curve indicated that kinase assays were conducted at near-maximum concentrations of ATP, and that ATP depletion is not likely to be a factor in the results obtained.

c-src phosphorylation

Peptide mapping indicated a readily-detectable (approximately 3-4 fold) increase in tyrosine phosphorylation of a 10 kDa cyanogen bromide peptide, corresponding to the autophosphorylation site of c-src, in ErbB2-associated src (compared with total cellular c-src). Similarly, and a corresponding decrease in tyrosine phosphorylation of a 4 kDa peptide, which corresponds to the inhibitory tyrosine at position 527 (Nada et al., 1993) was observed (Figure 2).

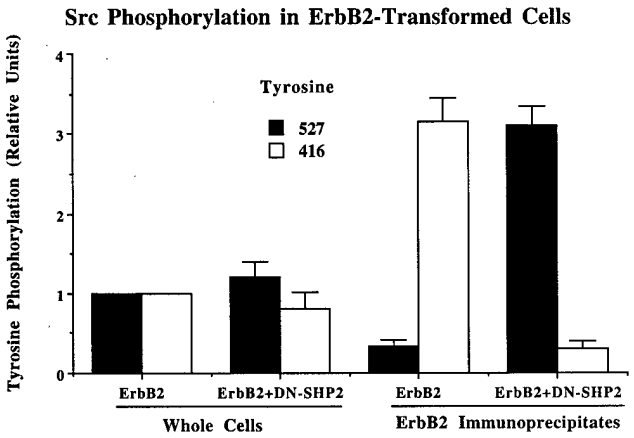

Figure 2. Phosphorylation site of c-src in vivo in mammary epithelial cells transfected with ErbB2 or ErbB2 + dominant negative SHP2. .

c-src Expression

Total c-src content of 184.A1 cells, ErbB2 transformed cells and ras transformed cells was similar, indicating that changes observed above were likely to be due to activation of existing c-src, not increased c-src expression.

Other src-related kinases

To date, we have not observed activation of lyk, lyn or fyn in response to ErbB2 transformation.

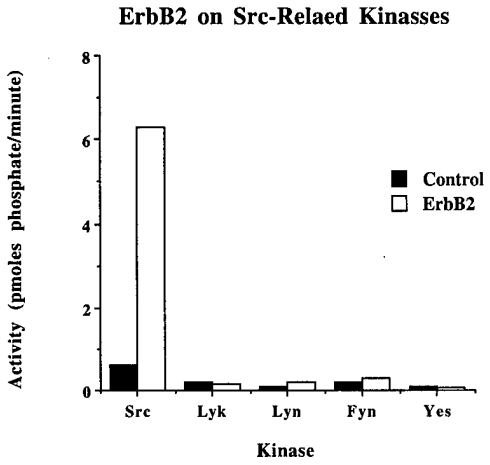

Figure 3. Activity of src-related kinases in vector-transfected (Control) and ErbB2-transformed 184.1 cells. Kinase was immunoprecipitated from indicated cells and activity determined as described in Methods. Mean of 3 determinations.

Other Cell Lines

Most of the work to date has been on 184.A1 cells and their transformed derivatives. However, we have also transformed other cell lines with ErbB2 (some in preliminary studies described in the grant proposal). These include NMuMG (a nontumorigenic mouse mammary epithelial line) and MCF 10A (a non-tumorigenic human mammary epithelial cell line). In both of these lines, ErbB2 increases c-src activity with little or no change in c-src protein content of cells. Although baseline levels of c-src activity vary somewhat among cell lines, the fold induction by ErbB2 was similar among lines (Figure 4).

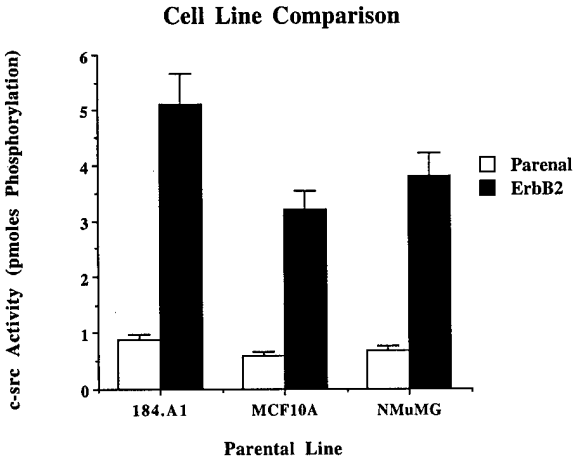

Figure 4. Activation of c-src by ErbB2 transformation in various cell lines. Mean ± SEM of 3 determinations.

Expression and Activity of CSK

Since a possible mechanism by which ErbB2 could increase c-src activity is to decrease expression or activity of C-terminal Src Kinase (CSK), we examined the expression and activity of CSK in ErbB2 and Ras transformed cell lines. Ras had no significant effect on CSK level, whereas ErbB2 significantly increased CSK expression level (Figure 5). These effects were paralleled by a similar increase in CSK activity. When corrected for differences in CSK content, there was no effect of ErbB2 on specific activity of CSK. Thus, it appears that ErbB2 does not activate c-src by decreasing the rate at which Y527 is phosphorylated.

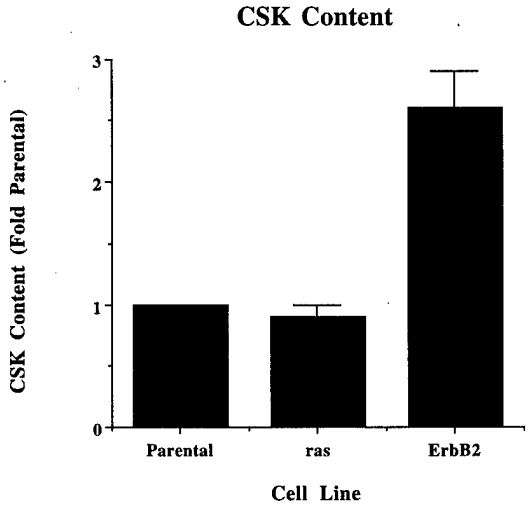

Figure 5. Effect of ErbB2 on CSK expression in 184.1 cells transformed with ErbB2 or ras.

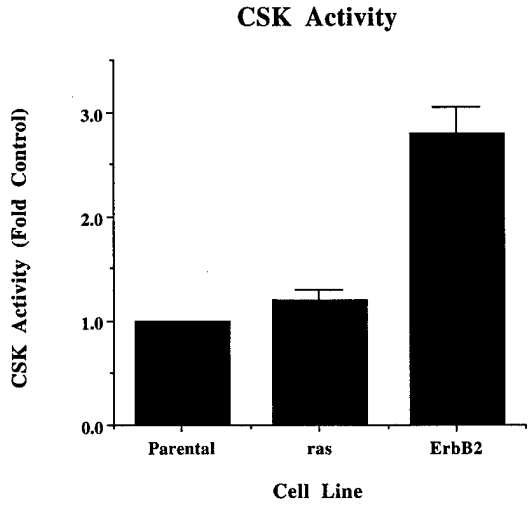

Figure 6. Activity of CSK in 184.1 cells transformed with ErbB2 or ras.

Total Phosphatase Activity

To assess total phosphatase activity in various cell lines, two substrate peptides were used: 312, corresponding to Y416, the autophosphorylation site of c-src, and 301, corresponding to Y527, the CSK phosphorylated site of c-src. Substantially higher activity toward peptide 301 was observed, but there was no difference among cell lines in this activity. Lower activity was observed toward peptide 312, but this activity was slightly higher in ErbB2 transformed cell lines than in other lines.

Membrane associated Phosphatase Activity

Cell membranes and cytosol prepared from control, ras or ErbB2 transformed cells were assessed for phosphatase activity toward peptide 301 and 312. There was no difference among cell lines in peptide 312 dephosphorylation in either membrane or cytosolic fractions. In contrast, the membrane fraction of ErbB2 transformed cells exhibited substantially greater activity toward peptide 301 than other cell lines. However, the cytosolic fraction showed little difference in activity among the cell lines. These results indicated an increased membrane associated phosphatase activity with activity selective for Y527 of c-src. Further examination of the activity indicated that it was linear over the amount of membrane protein used and over time. In addition, the activity was dependent on substrate, and near Vmax conditions were used in the standard assay.

ErbB2 associated phosphatase activity

Phosphatase activity in ErbB2 immunoprecipitates was assessed essentially as for membrane fractions. These studies indicated that ErbB2 was physically associated with a phosphatase with selectivity toward Y527 of CSK, and that the amount of this enzyme that co-precipitated with ErbB2 was dramatically increased (about 9 fold) in cells transfected with ErbB2 (Figure 7). However, it should be noted that ErbB2 transformed cells express 9-10 fold more ErbB2, so that the increased ErbB2 association may be due to the increased amount of ErbB2, not a specific increase in phosphatase-ErbB2 association in transformed cells.

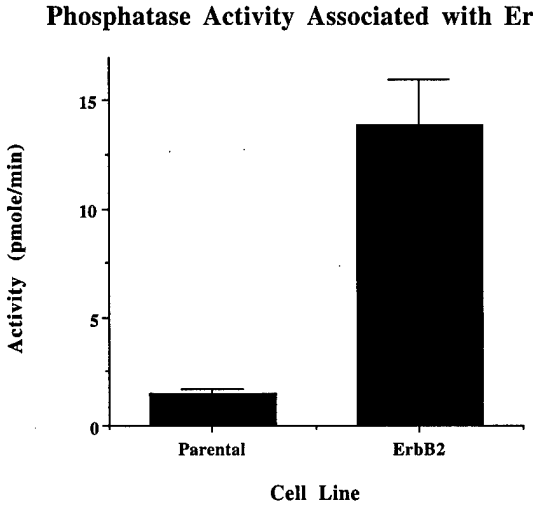

Figure 7. Phosphatase activity toward the CSK phosphorylation site of src in ErbB2 immunoprecipitates prepared from parental and ErbB2 transformed human mammary epithelium.

Specific Phosphatases

Western blot analysis indicated that the cell lines examined expressed detectable levels of PTP1B and LAR, and these were increased by transformation. The cells expressed little SHP1 and SHP1 content was not affected by transformation. The cells expressed substantial amounts of SHP2, and total SHP2 content was actually slightly lower in transformed cells (Figure 8). However, SHP2 associated with membrane fraction was substantially increased in ErbB2 transformed cells. In addition, the amount of SHP2 present in ErbB2 immunoprecipitates was increased about 10 fold, which is similar to the increase in ErbB2. These results, together with previous studies, suggest that SHP2 associates with ErbB2. Transformation with ErbB2 does not dramatically alter the specific association, but, due to greater ErbB2 expression, the total amount of SHP2 associated with ErbB2 is dramatically increased. This provides a plausible mechanism for ErbB2 activation of c-src, although that hypothesis has not yet been definitively tested.

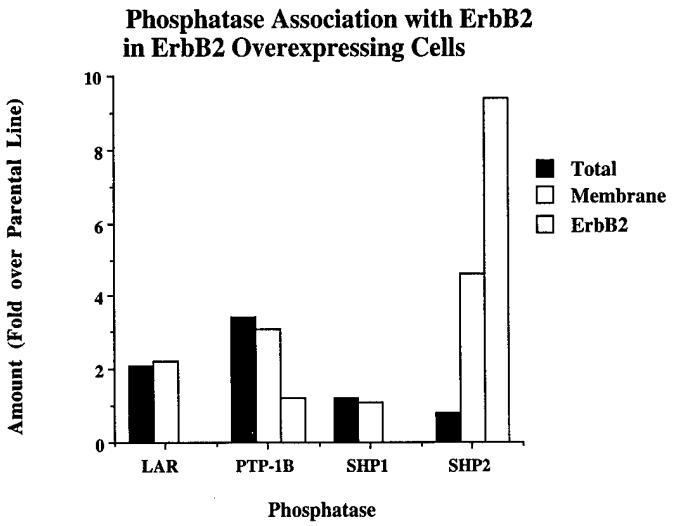

Figure 8. Expression level of specific phosphatases in ErbB2 transformed cell lines. Phosphatase content of crude cell lysates (Total), membrane preparations or ErbB2 immunoprecipitates were determined by Western analysis of parental and ErbB2 transformed human mammary epithelial cells and expressed as ErbB2 expression/Parental expression.

ErbB2-Dominant Negative c-src cell lines

In preliminary studies to characterize cell lines transfected with dominant negative c-src and ErbB2, the dominant negative mutant of c-src did not appear to inhibit cell proliferation on plastic. However, colony forming efficiency on soft agar was dramatically inhibited by dominant negative c-src when cells were transformed by ErbB2, but not by ras (Figure 9). Further evaluations of these cells are necessary, and scheduled for Year 3 of the project. These evaluations include replication of cell growth results, tumor formation in vitro, cell invasiveness assays and evaluations of c-src activity. However, the results to date indicate that c-src, although not necessary for cell proliferation, appears to be critical for ErbB2-mediated tumorigenic transformation.

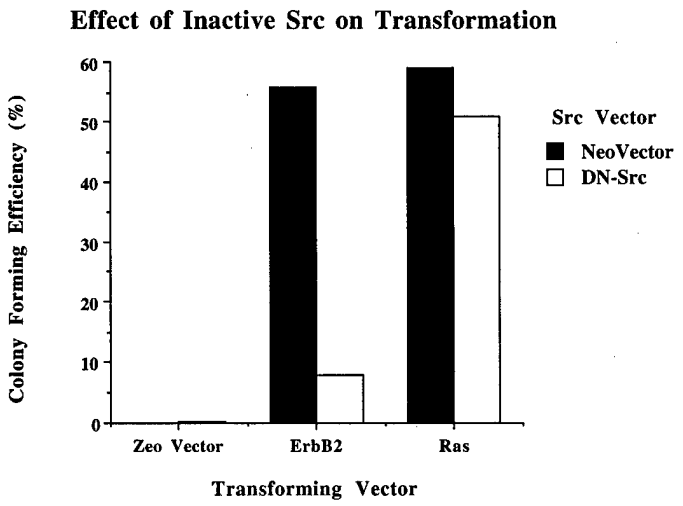

Figure 9. Colony forming efficiency of cells expressing combinations of ErbB2 and dominant negative src. Cells were transfected with a dominant negative kinase inactive src (DN Src) or vector alone (Neo Vector) and selected for G418 resistance. These cells were transfected with ErbB2, ras or vector alone (Zeo vector) and selected for Zeocin resistance. Cells were plated onto soft agar and colony forming efficiency deterined.

Co-transfection of ErbB2 and dominant negative SHP2

To further assess the role of SHP2 in ErbB2-induced activation of c-src, cells were co-transfected with ErbB2 and vector alone (control) or ErbB2 and dominant negative SHP2 (a truncation mutation lacking the phosphatase domain of SHP2). Expression of dominant negative SHP2 dramatically reduced the association of phosphatase activity with ErbB2 (Figure 10), suggesting that a major phosphatase associated with ErbB2 is SHP2. In additional studies, the impact of dominant negative SHP2 on c-src activation by ErbB2 was assessed. Expression of inactive SHP2 prevented the ErbB2-induced activation of c-src (Figure 3). Thus, a major pathway for ErbB2-activation of c-src appears to be activation of SHP2.

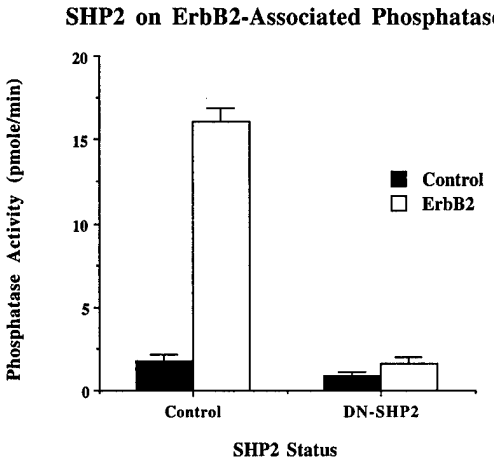

Figure 10. Effect of dominant negative SHP2 on ErbB2-associated phosphatase activity. Cells transfected with vector alone (Control) or dominant negative SHP2 (DN-SHP2) were transfected with vector (Control) or ErbB2. ErbB2 was immunoprecipitated and phosphatase activity determined as dephosphorylation of peptide corresponding to the C-terminus of C-src. Mean ± SEM of 4 experiments.

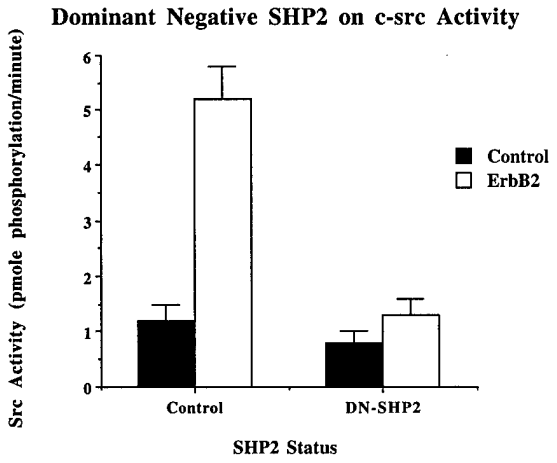

Figure 11. Effect of dominant negative SHP2 on ErbB2-induced c-src activity. Cells transfected with vector alone (Control) or dominant negative SHP2 (DN-SHP2) were transfected with vector (Control) or ErbB2. C-src activity was determined as described previously (Sheffield, 1998). Mean ± SEM of 4 experiments.

Dominant negative src alters tumor formation

Previously, dominant negative c-src was found to inhibit the ability of mammary epithelial cells to grow on soft agar. As an additional test of tumor formation, cells were xenografted to athymic (nude) mice. After 2 months, 12/12 control tumors (ErbB2 and vector alone) were detectable, whereas only 7/12 ErbB2-dominant negative src injections formed tumors. Furthermore, the growth rate of the control cells was significantly faster, resulting in larger final tumor weight (1.2 ± 0.3 g in controls vs 0.4 ± 0.2 g in dominant negative src expressing cells). These results indicate that c-src activation by ErbB2 appears to be necessary for tumor formation and development.

Discussion

Results indicated that c-src was activated by ErbB2 without increasing c-src expression. Standard assay validations indicated that this was due to a true increase in src activity. Results of these studies indicate that ErbB2 does not dramatically alter the amount of c-src in cells, but does increase c-src activity 4-6 fold. This appears to be associated with increased in vivo tyrosine phosphorylation on the autophosphorylation site of c-src (tyrosine 416) and decreased tyrosine 527 phosphorylation. This would appear to represent a mechanism of activation of c-src (Cooper and Howell, 1993).

There are several possible mechanisms by which ErbB2 could activate c-src. ErbB2 could decrease expression or activity of the kinase responsible for phosphorylating tyrosine 527 (most likely csk or c-terminal src kinase, Cooper and Howell, 1993). Our results suggest that this is not a likely mechanism, as ErbB2 expression was associated with increased, rather than decreased, CSK content and activity in mammary epithelium.

Alternatively, ErbB2 could increase the activity or level of phosphotyrosine phosphatases directed toward the C-terminus of c-src. Increased association of the phosphatase SHP2 with ErbB2 appears to be a plausible mechanism by which ErbB2

could activate src. In the present study, we determined that SHP2 was associated with ErbB2. Dominant negative SHP2 reduced phosphatase activity associated with ErbB2 and dramatically incrased src Y527 phosphorylation. These results suggest that SHP2 or a SHP2-like phosphatase is likely to regulate ErbB2-induced src activity.

A second major objective of the project is to determine the consequences of c-src activation by ErbB2. These studies suggest that c-src activation by ErbB2 has little effect on growth rates, but is an important factor in tumor formation.

KEY RESEARCH ACCOMPLISHMENTS

-ErbB2 transformation of mammary epithelial cells leads to increased activity of c-src without increasing expression of c-src.

-C-src phosphorylation pattern in ErbB2 transformed cells was consistent with dephosphorylation at Y527 in response to ErbB2.

-c-src dephosphorylation on Y527 does not appear to be due to decreased CSK content or activity.

-c-src dephosphorylation in response to ErbB2 appears to be mediated by increased SHP2 activity.

-c-src activation by ErbB2 appears to be necessary for ErbB2-induced tumorigenicity.

REPORTABLE OUTCOMES

Manuscripts

Sheffield, L.G. 1998. Role of c-src activation in ErbB2-induced transformation of human breast epithelium. Biochem. Biophys. Res. Commun. 250:27-31.

Sheffield, L.G., Smuga-Otto, K., Lewandowski, J.A., Vilhubner, K. ErbB2 activates of c-src via the phosphotyrosine phosphatase SHP2. Submitted.

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Sheffield, L.G. and J.A. Lewandowski. ErbB2 increases expression of C-terminal src kinase (CSK) in mammary epithelial cells. In Preparation.

Sheffield, L.G. 2000. Involvement of SHP2 in ErbB2-mediated tumorigenesis. Era of Hope Meeting, 2000, Atlanta, GA.

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CONCLUSIONS

Results to date indicate that overexpression of ErbB2 in nontumorigenic mammary epithelial cells increases activity, but not expression, of c-src. Furthermore, this effect is not mimicked by other transforming oncogenes. These results suggest that previous results in transgenic animals are likely to be due to direct effects on mammary epitheliaum, not to alterations in systemic physiology (such as hormone levels) or to altered epithelial stromal interactions. The results also suggest that src activation may lead to increased expression of csk. The role of this increased csk is not clear, but it may represent a negative feedback loop, as csk is generally considered a negative regulator of src. These results suggest that strategies to modify src activation may be useful in the development of breast cancer therapies.

Previous results indicate that ErbB2 increases activity of c-src in mammary epithelium. The present studies suggest that this induction of c-src activity is mediated by increased activity of a src-directed phosphatase that removes an inhibitory phosphate at Y527. SHP2 appears to be a leading candidate for the phosphatase. In addition, the activation of c-src by ErbB2 appears to play a critical role in inducing a tumor phenotype. These results that strategies to modify src activity or the activity of the src-directed phosphatase(s) may prove useful in modifying tumor progression.

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